EXHIBIT B

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Antithrombotic Therapy

The Effect of Dabigatran Plasma Concentrations and Patient Characteristics on the Frequency of Ischemic Stroke and Major Bleeding in Atrial Fibrillation Patients

The RE-LY Trial (Randomized Evaluation of Long-Term Anticoagulation Therapy)

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Objectives The goal of this study was to analyze the impact of dabigatran plasma concentrations, patient demographics, and aspirin (ASA) use on frequencies of ischemic strokes/systemic emboli and major bleeds in atrial fibrillation patients.

Background The efficacy and safety of dabigatran etexilate were demonstrated in the RE-LY (Randomized Evaluation of Long-Term Anticoagulation Therapy) trial, but a therapeutic concentration range has not been defined.

Methods In a pre-specified analysis of RE-LY, plasma concentrations of dabigatran were determined in patients treated with dabigatran etexilate 110 mg twice daily (bid) or 150 mg bid and correlated with the clinical outcomes of ischemic stroke/systemic embolism and major bleeding using univariate and multivariate logistic regression and Cox regression models. Patient demographics and ASA use were assessed descriptively and as covariates.

Results Plasma concentrations were obtained from 9,183 patients, with 112 ischemic strokes/systemic emboli (1.3%) and 323 major bleeds (3.8%) recorded. Dabigatran levels were dependent on renal function, age, weight, and female sex, but not ethnicity, geographic region, ASA use, or clopidogrel use. A multiple logistic regression model (c-statistic 0.657, 95% confidence interval [CI]: 0.61 to 0.71) showed that the risk of ischemic events was inversely related to trough dabigatran concentrations ($p = 0.045$), with age and previous stroke (both $p < 0.0001$) as significant covariates. Multiple logistic regression (c-statistic 0.715, 95% CI: 0.69 to 0.74) showed major bleeding risk increased with dabigatran exposure ($p < 0.0001$), age ($p < 0.0001$), ASA use ($p < 0.0003$), and diabetes ($p = 0.018$) as significant covariates.

Conclusions Ischemic stroke and bleeding outcomes were correlated with dabigatran plasma concentrations. Age was the most important covariate. Individual benefit–risk might be improved by tailoring dabigatran dose after considering selected patient characteristics. (Randomized Evaluation of Long Term Anticoagulant Therapy [RE-LY] With Dabigatran Etexilate; NCT00262600) (J Am Coll Cardiol 2014;63:321–8) a 2014 by the American College of Cardiology Foundation

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Abbreviations and Acronyms

AF = atrial fibrillation

ASA = aspirin

bid = twice daily

CAD = coronary artery disease

CI = confidence interval

CrCl = creatinine clearance

DE = dabigatran etexilate

DE 110 = dabigatran etexilate 110 mg twice daily

DE 150 = dabigatran etexilate 150 mg twice daily

PK = pharmacokinetic(s)

SEE = systemic embolic event(s)

Dabigatran etexilate (DE), a new, oral, direct thrombin inhibitor was shown in the RE-LY (Randomized Evaluation of Long-Term Anticoagulation Therapy) trial to be as effective (110 mg twice daily [bid], DE 110) or more effective (150 mg bid, DE 150) than warfarin, when given as a fixed dose without laboratory monitoring for prevention of stroke and systemic embolism in atrial fibrillation (AF) patients (1–3). The low dose of DE was associated with significantly less bleeding than both warfarin and the high dose of DE. Compared with DE 110, exposure to dabigatran was increased by 36% with DE 150 (4), resulting in a 39% reduction in strokes/systemic emboli but at the cost of a 16% increase in major bleeding (1,2). Thus, the RE-LY trial established dose–response relationships for stroke prevention and bleeding with DE. However, there is large variability in the plasma concentrations achieved with any given dose, depending on absorption, renal function, and other patient factors (4–6). DE is a prodrug metabolized in the plasma and liver to the active moiety dabigatran (6). How much the risk of stroke or bleeding also varies across the concentration range has important implications for the benefit–risk ratio and the possibility to tailor the dose in individual patients. Currently, it is unknown whether there is a single concentration range where the balance between thromboembolic events and bleeding events is optimal for all AF patients.

The rates of stroke and major bleeding in DE-treated patients have been investigated across a variety of patient subgroups (1,7), but correlations of stroke and bleeding risk with individual plasma concentrations have not been presented. The aims of this pharmacokinetic (PK) analysis of the RE-LY trial were to explore the association between plasma concentrations and efficacy and safety outcomes, and to identify factors affecting the variability of plasma concentrations of dabigatran and their impact on outcome events in AF patients with an indication for oral anticoagulation.

Methods

The design and results of the RE-LY study have been previously published (1–3). Briefly, the primary objective of RE-LY was to establish the noninferiority of 2 doses of DE compared with warfarin for stroke prevention in patients with AF and 1 additional risk factor for stroke. This trial randomized 18,113 AF patients to 1 of 2 blinded doses of DE, DE 110 or DE 150, or to dose-adjusted warfarin titrated to an international normalized ratio of 2 to 3. Median follow-up was 2.0 years. The study, including the PK sampling, was approved by all appropriate national regulatory authorities and ethics committees. All patients provided written informed consent before study entry.

All patients with a valid blood sample and all ischemic stroke/systemic embolic events (SEE) or bleeding events that occurred on-treatment in these patients were included in the analysis, regardless of when the event occurred in relation to the sampling. Patients who were off-treatment at the time of sampling or the time of event were not included in the analysis. All primary and secondary outcome events were blindly and doubly adjudicated. The primary RE-LY study outcome was stroke or systemic embolism. Stroke type was subdivided into ischemic, hemorrhagic, and unknown. Definitions of RE-LY endpoints are described elsewhere (1,3). $CHADS_2$ score is a simple validated risk score that assigns 1 point for a history of congestive heart failure, hypertension, age 75 years or older, and diabetes mellitus and 2 points for a history of stroke or transient ischemic attack. CHA_2DS_2-VASc (8) and HAS-BLED (9) are other methods to assess risk for stroke or bleeding in AF patients.

PK and statistical methods. Peak and trough samples at steady state were collected for determination of drug concentration, activated partial thromboplastin time, and ecarin clotting time at 1-month post-randomization in all DE subjects who gave consent to participate, regardless of the time of any ischemic or bleeding event that occurred. Samples were collected from patients in all geographic regions. The frequencies generally approximated the number of recruited subjects. For trough concentrations, only samples collected within 10 to 16 h after the previous DE dose were considered. Similarly, for post-dose samples, only samples collected within 1 to 3 h after dosing were considered. Approximately 12% of samples were excluded from evaluation because of questionable records in blood sampling date/time or in administration date/time. Additional samples were taken at 3, 6, and 12 months from 2,143 subjects who participated in a PK substudy. For analyses reported here, the data were merged with the data from the substudy and analyzed together. The first trough and post-dose samples fulfilling the time-window rule were used from these subjects with multiple blood samples. The population PK (4) and pharmacodynamic data (activated partial thromboplastin time and ecarin clotting time) will be reported elsewhere.

Plasma concentrations of nonconjugated (free) dabigatran and of total dabigatran after alkaline cleavage of conjugates were determined by a validated high-performance liquid chromatography tandem mass spectrometry method at AAIPharma Deutschland GmbH & Co. KG, Neu-Ulm, Germany (10). Total dabigatran concentration was determined in all PK plasma samples, whereas nonconjugated dabigatran was determined in a subset (n ¼ 1,085) of samples in order to assess the prevalence of dabigatran acylglucuronides in this population.

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Logistic regression of events (ischemic stroke/SEE and major and minor bleeds) and associated log-transformed trough plasma concentrations was performed with and without covariates. If patients had more than 1 observation, the sample closest to the planned time after dose (2 h and 12 h) was used, regardless of the occurrence of an event. The best-fit model was defined based on the Akaike Information Criterion. The c-statistic for predictive value based on the receiver-operating characteristic curve was also calculated. The impact of the covariates age, sex, body mass index, creatinine clearance (CrCl), coronary artery disease (CAD), diabetes mellitus, prior stroke or transient ischemic attack, hypertension, heart failure, $CHADS_2$ score, concomitant aspirin (ASA) use, and concomitant clopidogrel use were investigated. Only covariates with $p < 0.2$ were retained in the model if the Akaike Information Criterion was improved. CrCl at baseline was calculated using the Cockroft-Gault equation.

The performance of the final logistic regression models for major bleeding and ischemic stroke/SEE was tested by predicting the event rates in the DE patients without plasma concentration measurements (n ¼ 3,584). For these patients, the validation dataset, the trough plasma concentrations, was predicted using the population PK model from RE-LY (4), and the individual stroke and bleed rates were calculated based on the final logistic regression models. Patients were sorted according to their calculated risk and divided into ascending deciles. For each decile, the mean predicted risks for an event and for no event were calculated and compared with observed rates. The predictive performance was evaluated by the Hosmer-Lemeshow test (Online Table 1, Online Fig. 1). Values of $p > 0.05$ indicate no significant difference between the predicted and observed event rates.

Post-dose concentrations provided no advantages compared with trough concentrations, so only the latter are reported in the logistic regression analyses. Cox regression spline analyses were performed to explore the covariate–concentration–response relationship without the restrictions of a covariate linear effect. Event rates for bleeding and thromboembolic events are for the entire duration of the trial (median duration 2.0 years). All statistical analyses were performed with SAS version 9.1 and higher (SAS Institute, Cary, North Carolina).

Results

Plasma concentrations of dabigatran were available from 9,183 and 8,449 patients for peak and trough measurements, respectively (76% and 70% of randomized patients). The geometric mean trough concentrations were 64.7 and 91.0 ng/ml for the DE 110 and DE 150 doses, respectively, with 10th to 90th percentiles of 28.2 to 155 ng/ml for DE 110 and 39.8 to 215 ng/ml for DE 150, a 5.2- to 5.5-fold range of variation (Table 1). Geometric mean trough concentrations were 41% higher for the DE 150 dose compared with the DE 110 dose, and peak concentrations were 38% higher, demonstrating dose proportionality. Peak/trough ratios were approximately 1.9:1 for both doses. When concentrations from each dose group were adjusted for dose, the normalized values (ng/ml/mg) were similar, again indicating dose proportionality. For all subsequent analyses, the data from both doses were pooled (Table 1).

Demographics. Table 2 shows the impact of demographic characteristics on dabigatran plasma concentrations. Only trough concentrations are shown; unless otherwise specified, similar relationships occurred for peak concentrations. Renal function (CrCl) was a key determinant of plasma concentrations. The subjects with moderate renal impairment (between 30 and 50 ml/min CrCl) showed a 2.29-fold higher trough concentration than the subjects with renal function undiminished by age (CrCl 80 ml/min). In the subjects with mild renal impairment (between 50 and 80 ml/min CrCl), the trough concentrations were 47% higher compared with the subjects with CrCl 80 ml/min (0.828 ng/ml/mg vs. 0.564 ng/ml/mg).

Concentrations of dabigatran increased with age, with a 68% increase in trough concentrations in patients age 75 years compared with those < 65 years. Renal function was highly correlated with age. Concentrations in female subjects were approximately 30% higher than those in male subjects.

Table 1 Plasma Concentrations of Total Dabigatran After Oral Administration of Dabigatran 110 or DE 150, and Dose-Normalized Concentrations

	DE 110 (ng/ ml)		DE 150 (ng/ ml)		DE 110 þ DE 150 (ng/ ml/ mg)	
	$C_{pre,ss}$ (n ¼ 4,227)	$C_{2,ss}$ (n ¼ 4,583)	$C_{pre,ss}$ (n ¼ 4,222)	$C_{2,ss}$ (n ¼ 4,600)	$C_{pre,ss}$ (n ¼ 8,449)	$C_{2,ss}$ (n ¼ 9,183)
gMean	64.7	126	91	175	0.795	1.54
gCV, %	79.9	75.3	81.9	74.1	80.9	74.7
Median	65.9	133	93	184	0.811	1.62
P10	28.2	52	39.8	74.3	0.349	0.648
P90	155	275	215	383	1.9	3.36
Min	1.15	1.07	1.04	2.3	0.00923	0.0129
Max	608	745	809	1,000	7.36	9.02

bid ¼ twice daily; $C_{2,ss}$ ¼ 2-h post-dose plasma concentration at steady state; $C_{pre,ss}$ ¼ pre-dose plasma concentration at steady state; DE ¼ dabigatran etexilate; DE 110 ¼ dabigatran etexilate 110 mg twice daily; DE 150 ¼ dabigatran etexilate 150 mg twice daily; gCV ¼ geometric coefficient of variation; gMean ¼ geometric mean; P10 ¼ 10th percentile; P90 ¼ 90th percentile.

Table 2 Dose-Normalized Plasma Concentrations (ng/ ml/ mg) of Dabigatran According to Demographic Characteristics in the RE-LY Trial

Characteristic	Measure	Subgroup 1	Subgroup 2	Subgroup 3	Subgroup 4
Sex		Male (n ¼ 5,524)	Female (n ¼ 2,925)		
	gMean	0.727	0.942	d	d
	gCV, %	78.2	69.3	d	d
	Median	0.736	0.967	d	d
	P10	0.324	0.419	d	d
	P90	1.7	2.21	d	d
Age, yrs		< 65 (n ¼ 1,466)	65 to < 75 (n ¼ 3,787)	75 (n ¼ 3,196)	
	gMean	0.586	0.749	0.982	d
	gCV, %	86	75.2	76	d
	Median	0.595	0.761	0.994	d
	P10	0.241	0.341	0.45	d
	P90	1.43	1.69	2.22	d
Weight, kg		< 50 (n ¼ 163)	50 to < 100 (n ¼ 6,852)	100 (n ¼ 1,433)	
	gMean	0.998	0.824	0.652	d
	gCV, %	83.8	80.6	77.1	d
	Median	1.01	0.84	0.66	d
	P10	0.41	0.365	0.281	d
	P90	2.63	1.94	1.56	d
CrCl, ml/ min		< 30 (n ¼ 18)	30 to < 50 (n ¼ 1,512)	50 to < 80 (n ¼ 3,937)	80 (n ¼ 2,690)
	gMean	1.87	1.29	0.828	0.564
	gCV, %	51.9	78	71.7	70.2
	Median	2.11	1.33	0.857	0.582
	P10	0.905	0.601	0.395	0.262
	P90	3.16	2.83	1.77	1.2
$CHADS_2$		0–1 (n ¼ 2,783)	2 (n ¼ 2,964)	3þ (> 2) (n ¼ 2,702)	
	gMean	0.688	0.808	0.908	d
	gCV, %	76.4	79.2	83.5	d
	Median	0.706	0.820	0.932	d
	P10	0.318	0.355	0.390	d
	P90	1.527	1.935	2.191	d
CHA_2DS_2-VASc		0–1 (n ¼ 282)	2 (n ¼ 1,663)	3þ (> 2) (n ¼ 6,504)	
	gMean	0.499	0.624	0.863	d
	gCV, %	82.4	73.5	79.6	d
	Median	0.513	0.636	0.887	d
	P10	0.218	0.284	0.380	d
	P90	1.115	1.376	2.10	d
HAS-BLED		0–1 (n ¼ 5,201)	2þ (> 1) (n ¼ 3,248)		
	gMean	0.701	0.972		
	gCV, %	76.5	81.3		
	Median	0.715	0.989		
	P10	0.316	0.415		
	P90	1.571	2.290		

Abbreviations as in Table 1.

Weight under 50 kg was associated with a geometric mean concentration of DE 21% higher than for subjects weighing 50 to 100 kg, and 53% higher than patients weighing 100 kg. Risk scores for stroke and bleeding were positively correlated with plasma concentrations. For ethnicity and geographic regional variation, there were no relevant differences in plasma concentrations (Online Tables 2 and 3).

Plasma concentrations and outcome events. On average, subjects who had a major bleeding event during the trial had higher trough and post-dose concentrations than subjects

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Table 3 Trough Concentrations of Dabigatran (ng/ ml/ mg) Grouped by Outcome Event Occurrence

	Major Bleed (n ¼ 323)	Any Bleed (n ¼ 2,319)	No Bleed (n ¼ 5,899)	Stroke/ SEE (þ) (n ¼ 129)	No Stroke/ SEE ( ) (n ¼ 8,250)	Stroke/ SEE/ Death (þ) (n ¼ 387)	No Stroke/ SEE/ Death ( ) (n ¼ 7,789)	CV Events* (þ) (n ¼ 391)	No CV Events ( ) (n ¼ 7,865)
gMean	113	86.9	72.8	76.6	76.5	88.5	75.4	87.8	75.6
gCV, %	79.8	81.4	84	84.1	83.9	84.7	83.3	89.5	83.1
Median	116	88.2	75.3	80.6	78.3	91.4	77.6	90.7	77.6
P10	46.7	35.7	30.7	26.4	32.1	33.1	31.8	31.2	32
P90	269	211	175	185	186	226	181	229	182

*Cardiovascular (CV) events include stroke, systemic embolism, pulmonary embolism, myocardial infarction, and vascular deaths.
(þ) ¼ with event on-treatment; ( ) ¼ without event; other abbreviations as in Table 1.

without bleeding events. The median trough and post-dose concentrations were 55% and 36% higher, respectively, in the subjects with a major bleeding event than those in the subjects without bleeding events. Median (10th to 90th percentiles) trough concentrations in 323 patients with major bleeds were 116 (46.7 to 269) ng/ml compared with 75.3 (30.7 to 175) ng/ml in 5,899 patients with no major bleed (Table 3). Plasma concentrations of dabigatran were higher in subjects with hemorrhagic stroke (n ¼ 11 with trough and 13 with peak measurements) than in the subjects (n ¼ 8,269 with trough and 8,971 with peak measurements) without hemorrhagic stroke (144 ng/ml vs. 78.4 ng/ml for trough and 241 ng/ml vs. 155 ng/ml for post-dose concentrations, respectively; $p > 0.05$). Median plasma concentrations in subjects with an ischemic stroke or SEE were not different from those without these events.

In multivariate analyses of dabigatran concentrations versus both ischemic and bleeding outcomes, CrCl and age had similar predictive values as covariates, but they were highly correlated. In logistic regression analyses with trough concentration and age in the model, CrCl was not significant.

The relationship between major bleeding and trough plasma concentrations was analyzed by logistic regression analyses with and without covariates. The best-fit model (c-statistic 0.715, 95% confidence interval [CI]: 0.69 to 0.74) included trough concentration ($p < 0.0001$), age ($p < 0.0001$), ASA use ($p ¼ 0.0003$), diabetes ($p ¼ 0.018$), clopidogrel use ($p ¼ 0.076$), sex ($p ¼ 0.078$), and CAD ($p ¼ 0.107$). Without plasma concentrations in the model, the c-statistic was 0.68. Figure 1 shows the risk of major bleeding and ischemic stroke/SEE versus trough concentration at different ages. The model showed an acceptable predictive performance of the bleeding events using the validation dataset (55 patients with major bleed events on-treatment, 3,464 patients without an event). The Hosmer-Lemeshow goodness-of-fit test was $p ¼ 0.082$, indicating no significant difference between observed and predicted events (Online Fig. 1).

In the multivariate analysis of ischemic stroke/SEE, there was an inverse relation between dabigatran trough concentrations and the probability of an event. The best-fit logistic regression model (c-statistic 0.657, 95% CI: 0.61 to 0.71) included age ($p < 0.0001$), previous stroke/transient ischemic attack ($p < 0.0001$), trough concentration ($p < 0.045$), and diabetes at baseline ($p ¼ 0.167$). Without plasma concentrations in the model, the c-statistic was 0.64. ASA use was not a significant covariate. For the validation

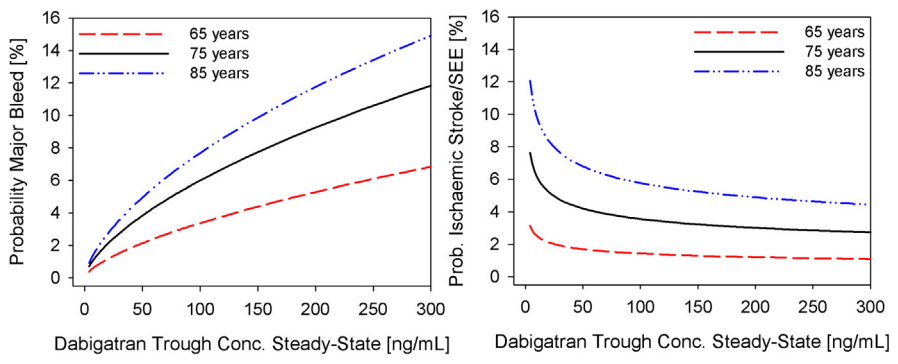

Figure 1 Probability of Clinical Outcomes Versus Dabigatran Plasma Concentrations

Major bleeding event (left) and ischemic stroke/ systemic embolic event (right) versus trough dabigatran plasma concentration in atrial fibrillation patients by age (65, 75, and 85 years). Covariates include sex, prior stroke, and diabetes. Conc. ¼ concentration.

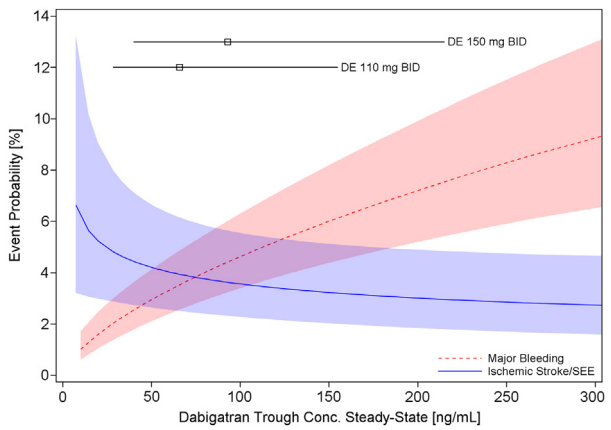

Figure 2 Probability of Major Bleeding Event and Ischemic Stroke/ SEE Versus Trough Plasma Concentration of Dabigatran

Calculated for 72-year-old male atrial fibrillation patient with prior stroke and diabetes. Lines and boxes at the top of the panel indicate median dabigatran concentrations in the RE-LY trial with 10th and 90th percentiles.
Conc. ¼ concentration; DE ¼ dabigatran etexilate; SEE ¼ systemic embolic event(s).

dataset (183 patients with ischemic stroke/SEE events on-treatment, 3,304 patients without event), the Hosmer-Lemeshow goodness-of-fit test was p ¼ 0.217, indicating a good fit. The slope of the concentration response curve for ischemic stroke/SEE was steepest at low concentrations (Figs. 1 and 2). A comparison of benefit and risk based on the logistic regression curves for major bleeding and ischemic stroke/SEE is shown in Figure 2 for a 72-year-old male patient with prior stroke and diabetes, together with the predicted median concentrations and 10th and 90th percentiles, for DE 110 and DE 150.

A Cox regression splines analysis of time to first major bleed with trough concentration, age, and $CHADS_2$ score as covariates showed that, compared with the median trough concentration of 88 ng/ml, adjusted for age and $CHADS_2$ score, the rate of major bleeding doubled at a concentration of 210 ng/ml (Fig. 3). Similarly, a Cox regression analysis of time to first ischemic stroke/SEE, with trough concentration, age, and $CHADS_2$ score as independent covariates, showed that the relative risk of ischemic stroke/SEE was increased by 50% at a concentration of 28 ng/ml compared with the risk at median trough concentrations (59 ng/ml) (Fig. 3).

Discussion

In this exposure response analysis from the RE-LY trial, the risks of major bleeding and ischemic stroke/SEE after dosing with DE 110 or DE 150 in patients with AF were related to trough concentrations of dabigatran. Significant factors affecting dabigatran plasma concentrations were age, CrCl, weight, and sex. Of these, all but weight are independent risk factors for stroke and bleeding risk in patients with AF.

The magnitude of the effect of dabigatran plasma concentrations on outcomes in AF patients in RE-LY depends strongly on demographic factors, most importantly increasing age. In multivariate analyses, age was the strongest independent covariate for prediction of a relationship between plasma concentration and outcome events, but age > 75 years is also directly related to the risk of strokes and bleeds in AF patients. As can be seen from Figure 1 and Table 1, plasma concentrations increase approximately 67% in patients age > 75 years compared with those < 65 years, but major bleed risk and stroke risk increase 2- to 3-fold. The effect of age on dabigatran exposure is likely due to the decreasing renal function in the elderly. Concentrations are increased 1.8-fold and 1.2-fold for patients with CrCl of 30 or 50 ml/min, respectively, compared with the median CrCl of 69 ml/min in RE-LY patients. In RE-LY, approximately

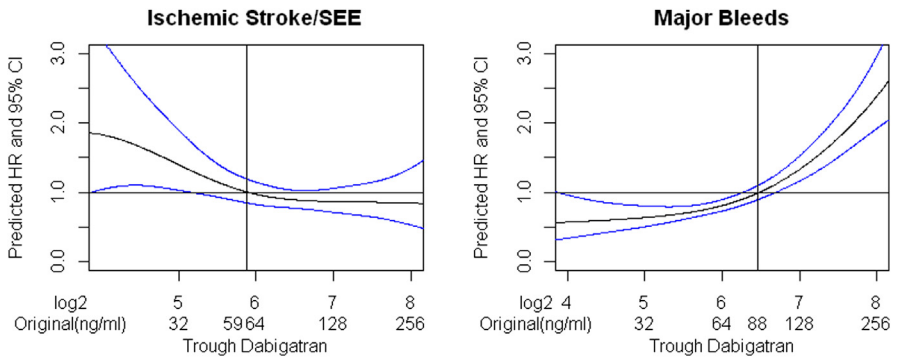

Figure 3 Cox Regression Analyses of Ischemic Stroke/ SEE and Major Bleeding Versus Trough Plasma Concentration of Dabigatran

The graphs show the hazard ratio (HR) of risk of event versus median trough dabigatran concentration (vertical line) in patients with median $CHADS_2$ score (2.0) and median age (71 years). Note log2 scale for concentrations. CI ¼ confidence interval; other abbreviations as in Figure 2.

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35% of patients ≥75 years had moderate renal dysfunction (CrCl 30 to 50 ml/min), and the median dabigatran concentration in patients ≥75 years was 39% higher than in patients <75 years (Table 2). Renal function, as measured by calculated CrCl, is no longer significant with age in the equation, although approximately 80% to 85% of dabigatran is excreted renally (6). Despite increased rates of major bleeding with decreasing renal function in DE patients, the relative risk of bleeding compared with warfarin did not change (7), suggesting that age and other factors in addition to anticoagulant effect or exposure play a role in the increased risk of stroke and bleeding seen in patients with renal dysfunction. Plasma concentrations also rose with increases in standard risk scores (Table 2). This likely reflects the negative impact of comorbidities and increasing age on renal clearance of dabigatran and further emphasizes the strong interaction between risk factors, plasma concentrations, and outcomes.

The concentration range achieved for either dose in RE-LY ranged over 5-fold for the 10th to 90th percentiles, with a large overlap of concentrations, approximately 70%, between the 2 doses. Given that the primary analyses of the whole population, without consideration of plasma levels, showed that the 2 doses of DE in RE-LY were effective and safe, this suggests that there is a wide therapeutic range. Nevertheless, there is a relevant effect of plasma concentrations on outcomes, with the c-statistic increasing from 0.64 to 0.66 for ischemic stroke/SEE and 0.68 to 0.72 for major bleeding. In some patients who are at the extremes of the concentration range and have 1 or more risk factors such as old age, reduced CrCl, or low body weight, better outcomes might be achieved by adjusting the dose.

Across the 10th to 90th percentile range of steady-state trough plasma concentrations achieved for the 150-mg bid dose, the overall risk of major bleeding during the trial ranges from approximately 2% to 7% (approximately 1.0% to 3.5% per year), with substantial impact of age and concomitant antiplatelet use, with sex, history of diabetes, and CAD also significant covariates in the model. Case reports of bleeding associated with extremely high DE plasma concentrations, over 2,000 ng/ml, have been reported (11). However, a lower dose to reduce bleeding must be weighed against the accompanying stroke risk, which increases with decreasing plasma concentrations. The risk of ischemic stroke over the same concentration range shows only a limited variation, for example, from 1.1% to 1.5% for a 72-year-old AF patient and 1.5% to 2.1% for an 80-year-old patient.

Because the risk of ischemic events is relatively constant for patients with higher plasma concentrations, reducing the daily dose in such patients may reduce the risk of bleeding without an appreciable loss of efficacy (Fig. 2). Older patients, those with multiple comorbidities, or those with reduced renal function may be appropriate candidates for a lower dose. Similarly, at concentrations below 28 ng/ml, for example, the 10th percentile for the 110-mg bid dose, the risk of ischemic events is high, but bleed risk is low. An upward adjustment of dose in patients with very low plasma concentrations should improve their risk–benefit ratio. However, this hypothesis should be tested in a controlled trial. The large majority of patients achieve a favorable balance of benefit and risk with a fixed dose of DE 110 or DE 150, guided by a consideration of patient characteristics.

Correlations of dabigatran plasma concentrations with bleeding and venous thromboembolism were previously used to support dose selection in an orthopedic surgery population (12). A dose response in AF patients has been previously demonstrated for DE for minor bleeding at doses from 100 to 600 mg/day (13). Long-term follow-up of these patients over 4 years has also demonstrated a dose response for major bleeding and for ischemic stroke and systemic embolism (14). Doses of 150 mg/day or less were inadequate for stroke prevention, and 600 mg/day led to high rates of major bleeding. However, none of these studies adjusted the dose based on plasma concentrations of dabigatran. Attempts to identify an optimal benefit–risk range for other antithrombotic medications based on clinical outcome events have met with mixed success for warfarin (15), ximelagatran (16), fondaparinux (17), and rivaroxaban (18).

Application of findings to clinical practice. In this RE-LY substudy, demographic characteristics played the strongest role in determining risk of clinical events. In patients at highest risk for events, such as the very elderly and/or those with poor renal function, an adjustment of DE dose to optimize exposure might improve the benefit–risk if they are at either extreme of the concentration range. However, an assay of dabigatran concentrations is not yet widely available, and at least in the United States, only the 150-mg bid dose is available except for a 75-mg bid dose in patients with severe renal failure. This substudy, therefore, can only serve as a basis for future endeavors in this area.

Study strengths and limitations. The size and scope of the plasma sampling of dabigatran in the RE-LY trial have permitted an analysis of the relationship between clinical outcomes and plasma concentrations of dabigatran, as well as the impact of demographic characteristics in patients with AF. Over 70% of the 12,091 patients randomized to DE had at least 1 plasma sample on-treatment. An assessment of benefit–risk is based on the primary safety and efficacy outcomes rather than laboratory measurement of anticoagulant activity.

This study has several limitations. Although the association of clinical outcomes with dabigatran concentrations was prespecified in the main study protocol, not all patients randomized to DE contributed a blood sample. This may have introduced some bias. Nevertheless, as demonstrated by goodness-of-fit estimates, predicted event rates in the patients who were not sampled agreed with the observed rates. Most of the blood sampling was carried out after 1 month on-treatment, at steady state, so early discontinuation of treatment for stroke or bleeding is not accounted for. Only patients who were on-treatment at the time of sampling and the time of the clinical event are included. There was no

temporal proximity of PK sampling and time of event. Medication compliance was not assessed in this analysis, which may introduce additional variability. Thus, intra-individual variability in plasma concentrations over time may blur the associations between dabigatran concentration and outcomes.

Conclusions

Both doses of DE in RE-LY were associated with a more than 5-fold variation in plasma concentrations, indicating a wide therapeutic range. Renal function was the predominant patient characteristic that determined plasma concentrations. Safety and efficacy outcomes were correlated with plasma concentrations of dabigatran, with age as the most important covariate. There is no single plasma concentration range that provides optimal benefit–risk for all patients. The balance between stroke risk and bleed risk varied with concentration, suggesting that there is a subset of AF patients who may improve their benefit–risk balance with DE by a tailoring of the dose in relation to patient characteristics.

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Key Words: atrial fibrillation · bleeding · dabigatran · pharmacokinetics · stroke.

APPENDIX

For a supplemental figure and tables, please see the online version of this article.